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Diagnostic value of CD103 expression in bronchoalveolar lymphocytes in sarcoidosis

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Summary
Background: Pulmonary sarcoidosis is frequently characterized by a CD4$^+$/CD8$^+$ ratio $\geq$3.5 in bronchoalveolar lavage fluid (BALF), although up to 40% of the cases present a normal or even decreased ratio, pointing out its variability and limitation as a diagnostic marker for sarcoidosis. Lung lymphocytes within the bronchial epithelium, the alveolar walls, and BALF express the integrin CD103. Our aim was to compare the expression of CD103 in BALF T-lymphocytes between sarcoidosis and other interstitial lung diseases (ILD) and to evaluate its relevance as a BALF diagnostic marker for sarcoidosis.
Methods: A total of 86 patients with ILD (mean age $\pm$ standard deviation, 42.6 $\pm$ 16.6 years; 60.5% female), who underwent BALF as part of their initial diagnostic work-up, were enrolled into 2 groups: sarcoidosis (n = 41) and other ILD (n = 45). Area under the receiver operating characteristic (ROC) curve (AUC) was used to describe the performance of CD103 for sarcoidosis diagnosis.
Results: Sarcoidosis patients presented a significantly reduced CD103 expression in BALF T-lymphocytes, more pronounced in the CD4$^+$ subset. The BALF CD103$^+$CD4$^+$/CD4$^+$ ratio for a cutoff point of 0.45 was associated with a better diagnostic performance for sarcoidosis (AUC: 0.86 [95% confidence interval (95% CI): 0.78–0.94]; sensitivity: 81%; specificity: 78%), even for those with a CD4$^+$/CD8$^+$ ratio <3.5 (AUC: 0.79 [95% CI: 0.64–0.93]; sensitivity: 75%; specificity: 78%).

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Conclusions: Assessment of CD103 expression in BALF $CD4^+$ T-lymphocytes may be a reliable tool for sarcoidosis diagnosis, independently of $CD4^+/CD8^+$ ratio, pointing out the relevance of evaluating the $CD103^+CD4^+/CD4^+$ ratio in the ILD diagnostic work-up.

Introduction

Sarcoidosis is the most common interstitial lung disease (ILD) in western world, with an estimated prevalence of 10—20 per 100,000 people.[1,2] It is a multisystem inflammatory disorder of unknown origin that commonly affects young adults. It is characterized by the accumulation of macrophages and $CD4^+$ T-lymphocytes in involved organs, with non-caseating granuloma formation, being the lungs, lymph nodes and skin the most frequently affected.[3,4]

The diagnosis of pulmonary sarcoidosis encloses a correct clinical setting, typical chest radiographic or high-resolution computed tomography (HRCT) features and a biopsy showing non-caseating granulomas.[3,5—7] Bronchoalveolar lavage fluid (BALF) is considered as a standard procedure in the diagnostic work-up of patients with ILD. In sarcoidosis the value of the BALF $CD4^+/CD8^+$ ratio to differentiate it from other ILD has been examined by several authors, which conclude that in patients with a typical clinical picture an elevated BALF $CD4^+/CD8^+$ ratio may support the diagnosis of sarcoidosis and obviate the need for confirmation by additional biopsy.[8—10] Those studies demonstrated that a $CD4^+/CD8^+$ ratio greater than 3.5 shows a high specificity of 93—96% for sarcoidosis, although the sensitivity is low, approximately 52—59%.[8,9,11,12] On the other hand, other studies[13] question the clinical usefulness of the BALF $CD4^+/CD8^+$ ratio, based on the observation that this ratio is highly variable. These authors found that only 42% of 86 patients with biopsy-proven sarcoidosis had a BALF ratio greater than 4.0, and that 12% had even an inverted ratio below 1.0, reflecting a predominance of $CD8^+$ T-lymphocytes.

Consequently, there has been a great interest in the investigation of other cellular markers with a reliable diagnostic accuracy for sarcoidosis. In this context, the expression of CD103 (integrin $\alpha_E\beta_7$) in BALF $CD4^+$ lymphocytes has been shown to be a promising candidate.[14]

Terminally differentiated mucosal intraepithelial T-lymphocytes express the integrin $\alpha_E\beta_7$, an adhesion molecule and homing receptor that binds to E-cadherin, a molecule vital for the adhesion and retention of epithelial cells.[15] In the lung, lymphocytes within the bronchial epithelium, the alveolar walls, and BALF express the integrin CD103,[16,17] although with a variable magnitude of expression.[18] In fact, a great variation of the CD103 expression in BALF $CD4^+$ and $CD8^+$ T-cells has been reported in ILD, suggesting a possible role of these subpopulations in the pathogenesis and differential diagnosis of some of these disorders.[19] Kolopp-Sarda et al. found that sarcoidosis was remarkably characterized by the lack of CD103 expression in the predominant $CD4^+$ subset.[14] The peripheral origin of $CD4^+$ BALF lymphocytes in sarcoidosis seems to be responsible for its lower expression of CD103.[14] These observations may be relevant for the diagnosis of sarcoidosis, namely through the analysis of $CD103^+CD4^+$ cell expression, along with the BALF $CD4^+/CD8^+$ and even BALF/peripheral blood ratios.[14,20]

Thus, we aimed to compare the expression of CD103 in BALF T-lymphocytes between patients with diagnosis of sarcoidosis and other ILD, and to evaluate its relevance as a BALF diagnostic marker for sarcoidosis.

Methods

Study population

A total of 86 patients with a confirmed ILD diagnosis (mean age ± standard deviation, 42.6 ± 16.6 years; 60.5% female), who underwent BALF as part of their initial diagnostic work-up, were enrolled in the study. Patients were divided into 2 groups for comparison effects, namely sarcoidosis ($n = 41$) and other ILD ($n = 45$).

Sarcoidosis diagnosis was based on a multimodality approach that combined clinical, radiological, and histological evaluation showing non-caseating granulomas, according to the American Thoracic Society/European Respiratory Society/World Association for Sarcoidosis and Other Granulomatous Disorders (ATS/ERS/WASOG) statement.[5] This diagnosis was also considered in those with clinical and radiological features typical of sarcoidosis associated with a BALF $CD4^+/CD8^+$ lymphocyte ratio $\geq$3.5. Chest radiographic staging was performed for all sarcoidosis patients according to Scadding criteria: stage 0 — normal ($n = 1$), stage I — mediastinal and bilateral hilar lymphadenopathy without lung involvement ($n = 14$), stage II — lymphadenopathy and lung involvement ($n = 21$), stage III — only lung involvement ($n = 0$), stage IV — lung fibrosis ($n = 5$).[5,21] A group of 22 patients had a diagnosis of hypersensitivity pneumonitis (HP), according to Schuyler et al.'s criteria.[22] The diagnosis of idiopathic interstitial pneumonia was based on surgical lung biopsy features or in accordance to the ATS/ERS International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias: idiopathic pulmonary fibrosis (IPF) ($n = 4$); non-specific interstitial pneumonia (NSIP) ($n = 3$); cryptogenic organizing pneumonia (COP) ($n = 1$).[23] Connective tissue disease-associated lung disorders were diagnosed according to international proposed criteria associated with HRCT scan and BALF features: systemic lupus erythematosus (SLE) ($n = 3$); rheumathoid arthritis ($n = 3$); scleroderma ($n = 2$).[24—26] Drug-induced lung disease (3 by rapamycin and 1 by capecitabine) and silicosis ($n = 3$) diagnosis were based on a compatible exposure history, radiological and BALF features.[27,28]

This study was approved by Ethics Committee of Centro Hospitalar de São João.

Bronchoalveolar lavage and flow cytometry

BAL was performed according to the ERS recommendations,[29] by flexible bronchoscopy at the time of diagnosis. Briefly, four aliquots of 50 mL sterile isotonic saline solution (37 °C) were instilled under fiberoptic bronchoscopy in the middle lobe and gently aspirated with a syringe (after each instillation). Recovered BALF was pooled (discarding the first aliquot), gauze filtered and the total cell numbers (Neubauer chamber) determined. Cell differentials were obtained by counting 500 cells on cytospin preparations stained with Wright-Giemsa. For phenotypic analysis, cells were centrifuged at 250 G for 10 min, washed twice, resuspended, and labeled with the following combination of monoclonal antibodies: anti-CD103-FITC, anti-CD8-Pe, anti-CD45-PercP-Cy.5 and anti-CD4-APC (Becton Dickinson Immunocytometry Systems, BDIS, San José, CA, USA). CD3, CD19, and CD56 expressions were also assessed in a parallel sample. Cells were acquired on a FACScan Flow Cytometer, using CellQuestPro Software (Beckton and Dickinson, BD Biosciences, San José, USA). Lymphocyte gating was based on forward scatter (FSC) versus side scatter (SSC). Additional gating was based on SSC versus CD45, CD4 and CD8 populations. BALF $CD4^+$, $CD8^+$, $CD103^+CD4^+$ and $CD103^+CD8^+$ T-lymphocytes were analyzed.

Statistical analysis

Data were described as mean and standard deviation or as median and interquartile range (IQR) for quantitative variables, and as counts and proportions. For comparison of quantitative variables the Student's *t*-test and the Mann—Whitney test were used according to variables distribution.

Sensitivity and specificity were calculated for a set of cutoff points of the various calculated forms of CD103 expression in BALF $CD4^+$ and $CD8^+$ T-lymphocytes.

The area under the receiver operating characteristic (ROC) curve (AUC), which plots sensitivity against 1 − specificity, was used to describe the diagnostic performance of those variables. Positive and negative predictive values (PPV and NPV) were also estimated.

Statistical analyses were performed using the Statistical Package for Social Sciences for Windows version 18 (SPSS; Chicago, IL, USA). Statistical significance was denoted by a *p*-value lower than 0.05 for all tests performed.

Results

Expression of CD103 in BALF T-lymphocytes

A significantly higher $CD4^+/CD8^+$ ratio in BALF was found for sarcoidosis patients compared to patients with other ILD (Table 1). The comparative analysis of the CD103 expression in the $CD4^+$ and $CD8^+$ T-lymphocyte subpopulations ($CD103^+CD4^+$ cells/μL; $CD103^+CD4^+$ %; $CD103^+CD4^+/CD4^+$; $CD103^+CD8^+$ cells/μL; $CD103^+CD8^+$ %; $CD103^+CD8^+/CD8^+$; $CD103^+CD4^+/CD103^+CD8^+$) between both groups (sarcoidosis versus other ILD) is represented in Table 1. As expected, in the sarcoidosis group, the absolute counts and percentages were significantly lower compared to the other group. Moreover, CD103 expression in the $CD4^+$ T-lymphocyte subset was lower than CD103 in $CD8^+$ T-cells.

As compared to other ILD, BALF $CD103^+CD4^+/CD4^+$ ratio, reflecting the relative number of $CD4^+$ T-lymphocytes that express CD103 within the total $CD4^+$ subpopulation, was also lower − 0.20 (IQR 0.25) versus 1.00 (IQR 1.50), $p < 0.001$ − in sarcoidosis patients (Table 1). This ratio was significantly higher in more advanced radiographic stages (stages ≤1: 0.10 (IQR 0.10) versus stages ≥2: 0.20 (IQR 0.43); $p = 0.044$).

Diagnostic criteria of BALF CD103 expression (sensitivity, specificity, cutoff levels)

Sets of cutoff points were established for the different values of CD103 expression in BALF T-lymphocytes, calculating their respective sensitivity and specificity values for sarcoidosis diagnosis (data available as supplementary material − Annexes 1 and 2). In accordance, and for the same cutoff ≤0.25, BALF $CD103^+CD4^+/CD4^+$ and

Table 1 Comparative analysis of T-lymphocyte subpopulations and $CD103^+$ expression between patients with sarcoidosis and other interstitial lung disease (ILD).

	Sarcoidosis ($n = 41$)	Other ILD ($n = 45$)	*p*-value
Lymphocytes (%)	34.8 (30.5)	53.0 (43.7)	0.014
$CD4^+$ (%)	79.4 (15.7)	38.0 (35.5)	<0.001
$CD8^+$ (%)	14.8 (11.7)	46.4 (33.1)	<0.001
$CD4^+/CD8^+$	5.7 (5.1)	0.9 (1.3)	<0.001
$CD103^+CD4^+$ (cells/μL)	5411 (19,179)	11,185 (43,215)	0.024
$CD103^+CD4^+$ (%)	11.9 (14.5)	32.6 (44.2)	<0.001
$CD103^+CD4^+/CD4^+$	0.20 (0.25)	1.00 (1.50)	<0.001
$CD103^+CD8^+$ (cells/μL)	2441 (6923.5)	42,646 (147357.5)	<0.001
$CD103^+CD8^+$ (%)	27.3 (46.6)	64.0 (55.6)	<0.001
$CD103^+CD8^+/CD8^+$	2.00 (2.35)	1.2 (1.45)	0.018
$CD103^+CD4^+/CD103^+CD8^+$	0.50 (0.60)	0.60 (0.50)	0.199

Quantitative variables are expressed as median and interquartile range (IQR).

Table 2 Sensitivity (Sn) and specificity (Sp) of the BALF $CD103^+CD4^+/CD4^+$ ratio for sarcoidosis diagnosis in the study population (left column), and in a subgroup of sarcoidosis with a BALF CD4/CD8 <3.5 (right column) all with histological confirmation, comparatively with the other ILD.

All sarcoidosis patients (n = 41)			Sarcoidosis with BALF $CD4^+/CD8^+$ <3.5 (n = 12)		
$CD103^+CD4^+/CD4^+$	Sn (%)	Sp (%)	$CD103^+CD4^+/CD4^+$	Sn (%)	Sp (%)
0.05	12	100	0.15	25	93
0.15	42	93	0.25	42	91
0.25	63	91	0.35	58	80
0.35	76	80	0.45	75	78
0.45	81	78	0.55	75	71
0.55	83	71	0.65	83	64
0.65	88	64	0.75	83	60
0.75	88	60	0.85	83	56
0.85	90	56	0.95	83	53
0.95	93	53	1.05	83	49

Sn, sensitivity; Sp, Specificity.

$CD103^+CD4^+/CD103^+CD8^+$ ratios were highly specific for sarcoidosis (specificity >90% for both), but with a low effect in sensitivity values (sensitivity for $CD103^+CD4^+/CD4^+$ <63% and for $CD103^+CD4^+/CD103^+$ $CD8^+$ <20%) (Table 2 and Annex 1).

ROC curves were performed in order to better evaluate the diagnostic value of CD103 BALF expression in sarcoidosis and to determine the cutoff values with the best sensibility and specificity relationship. Among the aforementioned variables, the $CD103^+CD4^+/CD4^+$ ratio, for a cutoff point of 0.45, was associated with a better diagnostic performance (sensitivity: 81%; specificity: 78%; PPV: 77%; NPV: 81%) (Table 3), as shown by ROC curves (Fig. 1a) and respective AUC (AUC: 0.86 [95% CI: 0.78—0.94]). In comparison, $CD103^+CD4^+/CD103^+CD8^+$ ratio, for the same cutoff value (0.45), despite a similar specificity, had a low sensitivity for sarcoidosis and consequently a lower AUC (sensitivity: 44%; specificity: 73%; PPV: 60%; NPV: 59%; AUC: 0.58 [95% CI: 0.46—0.70]) (Table 3 and Fig. 1c).

CD103 expression in sarcoidosis patients with BALF $CD4^+/CD8^+$ ratio < 3.5

Among the 41 sarcoidosis patients included, 12 (29.3%) presented a BALF $CD4^+/CD8^+$ <3.5 but with histological evaluation showing non-caseating granulomas. Similar statistical analysis was performed for this subgroup. In comparison with the others parameters, the $CD103^+CD4^+/CD4^+$ ratio (for the same cutoff point, 0.45, determined for the overall sarcoidosis group) revealed the best diagnostic performance, as shown by AUC magnitude (sensitivity: 75%; specificity: 78%; PPV: 47%; NPV: 92%; AUC: 0.79 [95% CI: 0.64—0.93]) (Table 4 and Fig. 1b).

Discussion

Sarcoidosis is characterized by an alveolar $CD4^+$ lymphocytosis, the effector cells involved in the T-helper 1 immune response, and consequently a high BALF $CD4^+/CD8^+$ ratio.[3,4,8–10] Although with a high specificity, an elevated $CD4^+/CD8^+$ ratio does not fully discriminate sarcoidosis from other ILD.[13] $CD4^+$ lymphocytosis may also be observed in other types of lung diseases,[30] and the sensitivity for sarcoidosis of an elevated $CD4^+/CD8^+$ ratio is seldom more than 50% in the studies available in literature.[8,9,11] Additionally, difficulties also rise in those cases of sarcoidosis with a normal or inverted $CD4^+/CD8^+$ ratio. Thus, other BALF cellular markers that may help in sarcoidosis differential diagnosis have been searched.

Table 3 Cutoff values, sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) for different bronchoalveolar lavage fluid (BALF) criteria evaluated for sarcoidosis diagnosis.

Criteria	Selected cutoff	Sn (%)	Sp (%)	PPV (%)	NPV (%)	AUC (CI 95%)
$CD103^+CD4^+$ (cells/μL)	7286	59	69	63	65	0.64 (0.53—0.76)
$CD103^+CD4^+$ (%)	17.85	71	80	76	75	0.75 (0.66—0.87)
$CD103^+CD4^+/CD4^+$	0.45	81	78	77	81	0.86 (0.78—0.94)
$CD103^+CD8^+$ (cells/μL)	19,954	98	62	70	97	0.84 (0.76—0.93)
$CD103^+CD8^+$ (%)	46.9	71	69	67	72	0.72 (0.61—0.83)
$CD103^+CD8^+/CD8^+$	1.35	71	60	62	69	0.65 (0.53—0.77)
$CD103^+CD4^+/CD103^+CD8^+$	0.45	44	73	60	59	0.58 (0.46—0.70)

Sn, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; AUC, area under curve; CI, confidence interval.

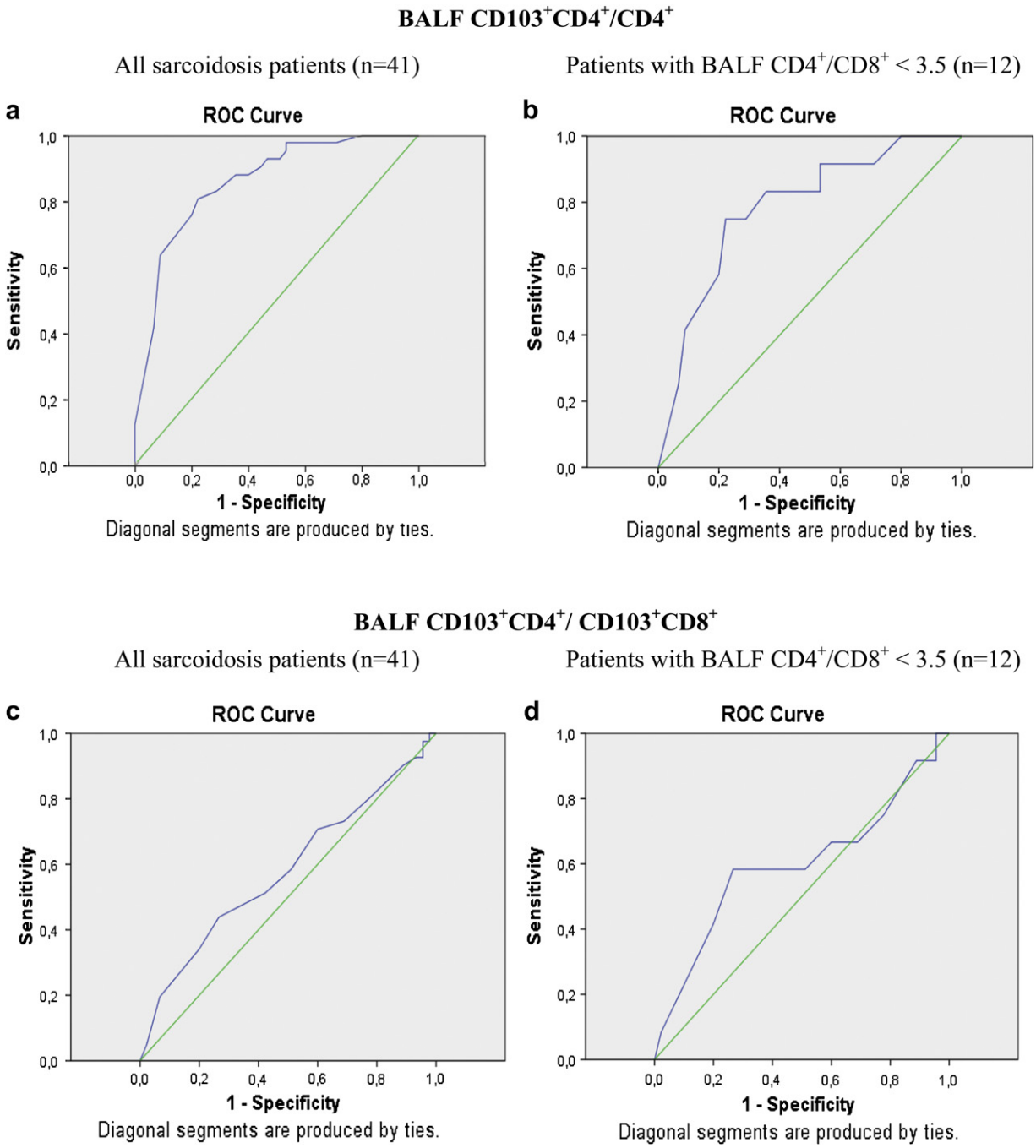

Figure 1 Receiver operating characteristic (ROC) curves of the bronchoalveolar lavage fluid (BALF) $CD103^{+}CD4^{+}/CD4^{+}$ and $CD103^{+}CD4^{+}/CD103^{+}CD8^{+}$ ratios in sarcoidosis patients independently of $CD4^{+}/CD8^{+}$ ratio (a, c) and for those with a $CD4^{+}/CD8^{+}$ ratio <3.5 (b, d), respectively.

In this context, the integrin CD103, expressed on intra-epithelial lymphocytes in mucosal areas, such as bronchi, has been studied in the diagnostic work-up of sarcoidosis. It has been shown that the relative amount of CD103-expressing T-cells in BALF differs in patients with ILD. This variation is predominantly seen in the $CD4^{+}$ T-cell population, as most of the $CD8^{+}$ T-cells express this integrin independently of the type of disease.[18] This was also shown in our study, as a low expression of $CD103^{+}$ was seen on $CD4^{+}$ rather than on $CD8^{+}$ BALF T-lymphocytes. Subsequently, a significantly lower BALF $CD103^{+}CD4^{+}/CD4^{+}$ ratio was also found in this group of patients. As other have suggested, this is corroborative with the concept that, for instance, lymphocytosis in HP results from the local expansion of mucosal lymphocytes, while in sarcoidosis, is the result of lymphocytes of non-mucosal origin. In fact, the relative absence of CD103 on $CD4^{+}$ BALF T-lymphocytes is consistent with a peripheral origin of these cells, favoring the hypothesis of redistribution from the peripheral blood and compartmentalization into the lung.[14,20,31,32] In fact, in

Table 4 Cutoff values, sensitivity (Sn), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) for different bronchoalveolar lavage fluid (BALF) criteria evaluated for sarcoidosis diagnosis for patients with BALF $CD4^+/CD8^+$ ratio <3.5 ($n = 12$; 29.3%).

Criteria	Selected cutoff	Sn (%)	Sp (%)	PPV (%)	NPV (%)	AUC (CI 95%)
$CD103^+CD4^+$ (cells/μL)	2951	50	84	46	86	0.69 (0.53—0.85)
$CD103^+CD4^+$ (%)	18.85	75	76	45	92	0.74 (0.57—0.91)
$CD103^+CD4^+/CD4^+$	0.45	75	78	47	92	0.79 (0.64—0.93)
$CD103^+CD8^+$ (cells/μL)	16,856	100	64	43	100	0.77 (0.65—0.89)
$CD103^+CD8^+$ (%)	27.65	50	82	43	86	0.64 (0.46—0.82)
$CD103^+CD8^+/CD8^+$	1.65	50	69	30	84	0.48 (0.29—0.68)
$CD103^+CD4^+/CD103^+CD8^+$	0.45	58	73	37	87	0.60 (0.39—0.80)

Sn, sensitivity; Sp, specificity; PPV, positive predictive value (PPV); NPV, negative predictive value; AUC, area under curve; CI, confidence interval.

our study BALF CD103 expression adequately differentiated sarcoidosis from the HP group, that showed a clearly higher BALF $CD103^+CD4^+/CD4^+$ ratio (data not shown).

Our results support the relevance to evaluate CD103 expression in BALF $CD4^+$ T-lymphocytes in sarcoidosis. Independently of the $CD4^+/CD8^+$ ratio, the BALF $CD103^+CD4^+/CD4^+$ ratio for a cutoff of 0.25 presented a high specificity (91%) for sarcoidosis diagnosis (Table 2). In order to characterize the diagnostic performance of this ratio, and to calculate the best relationship between sensitivity and specificity, we determined the cutoff value associated with the highest AUC value for this ratio, and for other CD103 expression values. In fact, the $CD103^+CD4^+/CD4^+$ ratio, for the cutoff 0.45, presented the highest AUC value, with a sensitivity and specificity of 81% and 78%, respectively. Taking into account these results, one can speculate that the $CD103^+CD4^+/CD4^+$ ratio ranging from 0.25 to 0.45 can be a useful tool for sarcoidosis diagnosis (Annex 1).

The potential diagnostic role of CD103 in sarcoidosis has been previously demonstrated by some authors. We cannot perform a direct comparison between our results and those found by these groups, as methodologies used differed. Kolopp-Sarda et al. have found that the combined use of the $CD4^+/CD8^+$ (≥ 2.5) and the $CD103^+/CD4^+$ ratio (<0.31) seems to be a promising new tool for sarcoidosis diagnosis, with sensitivity around 96%.[14] On the other hand, Heron et al. have already demonstrated, in a total of 119 patients with alveolar lymphocytosis (56 with pulmonary sarcoidosis), that the combined use of the $CD103^+CD4^+/CD4^+$ ratio (cutoff <0.2) with the $CD4^+/CD8^+$ (cutoff >3.0), or with the relative alveolitis $CD4^+/CD8^+$ BAL/periphery blood ratio (cutoff >2.0), provides a specific tool for discriminating sarcoidosis from other ILD (sensitivity: 66%; specificity: 89%; PPV: 82%; NPV: 74%).[20] As opposed to the aforementioned study, this last work, performed by the Dutch BAL working party, has some advantages, such as the inclusion of sarcoidosis patients with a $CD4^+/CD8^+$ <2.5, revealing the additional usefulness of CD103 in those patients without an apparent high $CD4^+$ alveolitis. Moreover, the application of the $CD103^+CD4^+/CD4^+$ ratio rather than the $CD103^+/CD4^+$ ratio, prevents the misleading effect of $CD8^+$ lymphocytes, that co-express CD103 in most lung diseases. Similarly, we did not evaluate CD103 global expression and we included patients with a great variability of BALF $CD4^+/CD8^+$ ratio. Among our 41 sarcoidosis patients, 12 presented a $CD4^+/CD8^+$ <3.5 (including 4 patients with an inverted $CD4^+/CD8^+$ <1). In this subgroup of patients, the $CD103^+CD4^+/CD4^+$ ratio for the same cutoff point (0.45), as it was established for the total sample, revealed a similar diagnostic performance, with an expected higher negative predictive value (92%). This emphasizes the potential diagnostic role of this ratio also in sarcoidosis patients with a normal or inverted $CD4^+/CD8^+$ ratio, obviating the need of more invasive diagnostic tools in order to obtain a histological confirmation. Our sample also differed in the diagnostic criteria applied for sarcoidosis, as we included not only patients with biopsy-proven diagnosis (the unique criteria applied in the former studies), but also, patients with a high $CD4^+/CD8^+$ ratio in the presence of a clinical/radiological picture typical of this diagnosis without histological confirmation. Consequently, we cannot compare the diagnostic values of $CD4^+/CD8^+$ ratio and CD103 expression for sarcoidosis, as independent or combined markers, as others have shown. Although this is a potential limitation of our study, we currently should not subject patients with a typical clinical, radiological and BALF features of sarcoidosis to invasive tests, in order to obtain a histological confirmation.

Additionally, the small number of patients with BALF $CD4^+/CD8^+$ ratio <3.5 could be considered a potential limitation of this study. Nevertheless, our results are in line with those previously described by Heron et al.,[20] concerning the diagnostic value of CD103 expression even in sarcoidosis patients with BALF CD4/CD8 ratio <3.5.

As described in the literature, higher values of the BALF $CD103^+CD4^+/CD4^+$ ratio were found in more advanced sarcoidosis radiographic stages.[19,20] $CD103^+$ cells are involved in fibrogenic inflammation, as shown by an analysis of single nucleotide polymorphisms spanning ITGAE, the gene encoding the αE (CD103) unit, suggesting that the genotypic analysis of this integrin may also be helpful in assessing sarcoidosis risk and prognosis.[17,33]

In conclusion, assessment of CD103 expression in BALF $CD4^+$ lymphocytes may be a reliable tool for sarcoidosis diagnosis, independently of $CD4^+/CD8^+$ ratio, pointing out the relevance of evaluating this marker on the ILD diagnostic work-up. In our study, through an extensive analysis of sensitivity and specificity of various forms of its expression we were able to redefine a BALF $CD103^+CD4^+/CD4^+$ lymphocyte ratio ≤ 0.45 as a good diagnostic marker for sarcoidosis, even in cases with $CD4^+/CD8^+$ ratio <3.5.

Conflict of interest

The authors have no conflict of interest.

Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rmed.2012.03.020.

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